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APPENDIX 3.X.X.

GUIDELINES FOR THE SURVEILLANCE
OF AVIAN INFLUENZA

Article 3.X.X.1.

Introduction

This Appendix defines the principles and provides a guide for the surveillance of notifiable avian influenza (NAI) in accordance with Appendix 3.8.1., applicable to countries seeking recognition for a declared NAI status, with or without the use of vaccination. This may be for the entire country, *zone* or *compartment*. Guidance for countries seeking free status following an *outbreak* and for the maintenance of NAI status are provided. This Appendix complements Chapter 2.7.12.

The presence of NAI in wild birds creates a particular problem. In essence, no country can declare itself free from avian influenza (AI) in wild birds. However, the definition of NAI in Chapter 2.7.12. refers to the infection in poultry only and this Appendix was developed under this definition.

The impact and epidemiology of NAI differ widely in different regions of the world and therefore it is impossible to provide specific guidelines for all situations. It is axiomatic that the surveillance strategies employed for demonstrating freedom from NAI at an acceptable level of confidence will need to be adapted to the local situation. Variables such as the frequency of contacts of poultry with wild birds, different biosecurity levels and production systems and the commingling of different susceptible species including domestic waterfowl require specific surveillance strategies to address each specific situation. It is incumbent upon the country to provide scientific data that explains the epidemiology of NAI in the region concerned and also demonstrates how all the risk factors are managed. There is therefore considerable latitude available to Member Countries to provide a well-reasoned argument to prove that absence of NAI virus (NAIV) infection is assured at an acceptable level of confidence.

Surveillance for NAI should be in the form of a continuing programme designed to establish that the country, *zone* or *compartment*, for which application is made, is free from NAIV infection.

Article 3.X.X.2.

General conditions and methods

- 1) A surveillance system in accordance with Appendix 3.8.1. should be under the responsibility of the *Veterinary Administration*. In particular:
 - a) a formal and ongoing system for detecting and investigating *outbreaks of disease* should be in place;
 - b) a procedure should be in place for the rapid collection and transport of samples from suspect cases of NAI to a laboratory for NAI diagnosis as described in the *Terrestrial Manual*;
 - c) a system for recording, managing and analysing diagnostic and surveillance data should be in place.

The NAI surveillance programme should:

- a) include an early warning system throughout the production, marketing and processing chain for reporting suspicious cases. Farmers and workers, who have day-to-day contact with poultry, as well as diagnosticians, should report promptly any suspicion of NAI to the *Veterinary Authority*. They should be supported directly or indirectly (e.g. through private veterinarians or *veterinary para-professionals*) by government information programmes and the *Veterinary Administration*. All suspected cases of NAI should be investigated immediately. Where suspicion cannot be resolved by epidemiological and clinical investigation, as is frequently the case with LPNAI virus infections, samples should be taken and submitted to an *approved laboratory*. This requires that sampling kits and other equipment are available for those responsible for surveillance. Personnel responsible for surveillance should be able to call for assistance from a team with expertise in NAI diagnosis and control. In cases where potential public health implications are suspected, notification to the appropriate public health authorities is essential;
- b) implement, when relevant, regular and frequent clinical inspection, serological and virological testing of high-risk groups of animals, such as those adjacent to an NAI infected country, *zone* or *compartment*, places where birds and poultry of different origins are mixed, such as live bird markets, poultry in close proximity to waterfowl or other sources of NAIIV.

An effective surveillance system will periodically identify suspicious cases that require follow up and investigation to confirm or exclude that the cause of the condition is NAIIV. The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably. Applications for freedom from NAIIV infection should, in consequence, provide details of the occurrence of suspicious cases and how they were investigated and dealt with. This should include the results of laboratory testing and the control measures to which the animals concerned were subjected during the investigation (quarantine, movement stand-still orders, etc.).

Article 3.X.X.3.

Surveillance strategies

The target population for surveillance aimed at identification of *disease* and *infection* should cover all the susceptible poultry species within the country, *zone* or *compartment*. Active and passive surveillance for NAI should be ongoing. The frequency of active surveillance should be at least every 6 months. Surveillance should be composed of random and targeted approaches using virological, serological and clinical methods.

The strategy employed may be based on randomised sampling requiring surveillance consistent with demonstrating the absence of NAIIV infection at an acceptable level of confidence. The frequency of sampling should be dependent on the epidemiological situation. Random surveillance is conducted using serological tests described in the *Terrestrial Manual*. Positive serological results should be followed up with virological methods.

Targeted surveillance (e.g. based on the increased likelihood of *infection* in particular localities or species) may be an appropriate strategy. Virological and serological methods should be used concurrently to define the NAI status of high risk populations.

A country should justify the surveillance strategy chosen as adequate to detect the presence of NAIIV infection in accordance with Appendix 3.8.1. and the prevailing epidemiological situation. It may, for example, be appropriate to target clinical surveillance at particular species likely to exhibit clear clinical signs (e.g. chickens). Similarly, virological and serological testing could be targeted to species that may not show clinical signs (e.g. ducks).

If a Member Country wishes to declare freedom from NAIV infection in a specific *zone* or *compartment*, the design of the survey and the basis for the sampling process would need to be aimed at the population within the *zone* or *compartment*.

For random surveys, the design of the sampling strategy will need to incorporate epidemiologically appropriate design prevalence. The sample size selected for testing will need to be large enough to detect *infection* if it were to occur at a predetermined minimum rate. The sample size and expected disease prevalence determine the level of confidence in the results of the survey. The applicant country must justify the choice of design prevalence and confidence level based on the objectives of surveillance and the epidemiological situation, in accordance with Appendix 3.8.1. Selection of the design prevalence in particular clearly needs to be based on the prevailing or historical epidemiological situation.

Irrespective of the survey approach selected, the sensitivity and specificity of the diagnostic tests employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated for the vaccination/infection history and the different species in the target population.

Irrespective of the testing system employed, surveillance system design should anticipate the occurrence of false positive reactions. If the characteristics of the testing system are known, the rate at which these false positives are likely to occur can be calculated in advance. There needs to be an effective procedure for following up positives to ultimately determine with a high level of confidence, whether they are indicative of infection or not. This should involve both supplementary tests and follow-up investigation to collect diagnostic material from the original sampling unit as well as flocks which may be epidemiologically linked to it.

The principles involved in surveillance for *disease/infection* are technically well defined. The design of surveillance programmes to prove the absence of NAIV infection/circulation needs to be carefully followed to avoid producing results that are either insufficiently reliable to be accepted by the OIE or international trading partners, or excessively costly and logistically complicated. The design of any surveillance programme, therefore, requires inputs from professionals competent and experienced in this field.

1) Clinical surveillance

Clinical surveillance aims at the detection of clinical signs of NAI at the flock level. Whereas significant emphasis is placed on the diagnostic value of mass serological screening, surveillance based on clinical inspection should not be underrated. Monitoring of production parameters, such as increased mortality, reduced feed and water consumption, presence of clinical signs of a respiratory disease or a drop in egg production, is important for the early detection of NAIV infection. In some cases, the only indication of LPNAIV infection may be a drop in feed consumption or egg production.

Clinical surveillance and laboratory testing should always be applied in series to clarify the status of NAI suspects detected by either of these complementary diagnostic approaches. Laboratory testing may confirm clinical suspicion, while clinical surveillance may contribute to confirmation of positive serology. Any sampling unit within which suspicious animals are detected should be classified as infected until evidence to the contrary is produced.

Identification of suspect flocks is vital to the identification of sources of NAIV and to enable the molecular, antigenic and other biological characteristics of the virus to be determined. It is essential that NAIV isolates are sent regularly to the regional Reference Laboratory for genetic and antigenic characterization.

2) Virological surveillance

Virological surveillance using tests described in the *Terrestrial Manual* should be conducted:

- a) to monitor at risk populations;
- b) to confirm clinically suspect cases;
- c) to follow up positive serological results;
- d) to test 'normal' daily mortality, to ensure early detection of infection in the face of vaccination or in *establishments* epidemiologically linked to an *outbreak*.

3) Serological surveillance

Serological surveillance aims at the detection of antibodies against NAIV. Positive NAIV antibody test results can have four possible causes:

- a) natural infection with NAIV;
- b) vaccination against NAI;
- c) maternal antibodies derived from a vaccinated or infected parent flock are usually found in the yolk and can persist in progeny for up to 4 weeks;
- d) positive results due to the lack of specificity of the test.

It may be possible to use serum collected for other survey purposes for NAI surveillance. However, the principles of survey design described in these guidelines and the requirement for a statistically valid survey for the presence of NAIV should not be compromised.

The discovery of clusters of seropositive flocks may reflect any of a series of events, including but not limited to the demographics of the population sampled, vaccinal exposure or infection. As clustering may signal infection, the investigation of all instances must be incorporated in the survey design. Clustering of positive flocks is always epidemiologically significant and therefore should be investigated.

If vaccination cannot be excluded as the cause of positive serological reactions, diagnostic methods to differentiate antibodies due to infection or vaccination should be employed.

The results of random or targeted serological surveys are important in providing reliable evidence that no NAIV infection is present in a country, *zone* or *compartment*. It is therefore essential that the survey be thoroughly documented.

3) Virological and serological surveillance in vaccinated populations

The surveillance strategy is dependent on the type of vaccine used. The protection against AI is haemagglutinin subtype specific. Therefore, two broad vaccination strategies exist: 1) inactivated whole AI viruses, and 2) haemagglutinin expression-based vaccines.

In the case of vaccinated populations, the surveillance strategy should be based on virological and/or serological methods and clinical surveillance. It may be appropriate to use sentinel birds for this purpose. These birds should be unvaccinated, AI virus antibody free birds and clearly and permanently identified. The interpretation of serological results in the presence of vaccination is described in 3.X.X.6.

Article 3.X.X.4.

Documentation of NAI or HPNAI free status

1) Countries declaring freedom from NAI or HPNAI for the country, zone or compartment

In addition to the general conditions described in Chapter 2.7.12. of the *Terrestrial Code*, a Member Country declaring freedom from NAI for the entire country, or a *zone* or a *compartment* should provide evidence for the existence of an effective surveillance programme. The strategy and design of the surveillance programme will depend on the prevailing epidemiological circumstances and should be planned and implemented according to general conditions and methods described in this Appendix, to demonstrate absence of NAIV infection, during the preceding 12 months in susceptible poultry populations (vaccinated and non-vaccinated). This requires the support of a laboratory able to undertake identification of NAIV infection through virus detection and antibody tests described in the *Terrestrial Manual*. This surveillance may be targeted to poultry population at specific risks linked to the types of production, possible direct or indirect contact with wild birds, multi-age flocks, local trade patterns including live bird markets, use of possibly contaminated surface water, and the presence of more than one species on the holding and poor biosecurity measures in place.

2) Additional requirements for countries, zones or compartments that practise vaccination

Vaccination to prevent the transmission of HPNAI virus may be part of a disease control programme. The level of flock immunity required to prevent transmission will depend on the flock size, composition (e.g. species) and density of the susceptible poultry population. It is therefore impossible to be prescriptive. The vaccine must also comply with the provisions stipulated for NAI vaccines in the *Terrestrial Manual*. Based on the epidemiology of NAI in the country, *zone* or *compartment*, it may be that a decision is reached to vaccinate only certain species or other poultry subpopulations.

In all vaccinated flocks there is a need to perform virological and serological tests to ensure the absence of virus circulation. The use of sentinel poultry may provide further confidence of the absence of virus circulation. The tests have to be repeated at least every 6 months or at shorter intervals according to the risk in the country, *zone* or *compartment*.

Evidence to show the effectiveness of the vaccination programme should also be provided.

Article 3.X.X.5.

Countries, zones or compartments re-declaring freedom from NAI or HPNAI following an outbreak

In addition to the general conditions described in Chapter 2.7.12., a country re-declaring for country, *zone* or *compartment* freedom from NAI or HPNAI virus infection should show evidence of an active surveillance programme depending on the epidemiological circumstances of the *outbreak* to demonstrate the absence of the *infection*. This will require surveillance incorporating virus detection and antibody tests described in the *Terrestrial Manual*.

A Member Country declaring freedom of country, *zone* or *compartment* after an *outbreak* of NAI or HPNAI (with or without vaccination) should report the results of an active surveillance programme in which the NAI or HPNAI susceptible poultry population undergoes regular clinical examination and active surveillance planned and implemented according to the general conditions and methods described in these guidelines. The surveillance should at least give the confidence that can be given by a randomized representative sample of the populations at risk.

Article 3.X.X.6.

NAI free establishments within HPNAI free compartments

The declaration of NAI free *establishments* requires the demonstration of absence of NAIV infection. Birds in these *establishments* should be randomly tested using virus detection or isolation tests, and serological methods, following the general conditions of these guidelines. The frequency of testing should be based on the risk of infection and at a maximum interval of 21 days.

Article 3.X.X.7.

The use and interpretation of serological and virus detection tests

Poultry infected with NAI virus produce antibodies to haemagglutinin (HA), neuraminidase (NA), nonstructural proteins (NSPs), nucleoprotein/matrix (NP/M) and the polymerase complex proteins. Detection of antibodies against the polymerase complex proteins will not be covered in this Appendix. Tests for NP/M antibodies include direct and blocking ELISA, and agar gel immunodiffusion (AGID) tests. Tests for antibodies against NA include the neuraminidase inhibition (NI), indirect fluorescent antibody and direct ELISA tests. For the HA, antibodies are detected in haemagglutination inhibition (HI) and neutralization (SN) tests. The HI test is reliable in avian species but not in mammals. The SN test can be used to detect subtype specific antibodies to the haemagglutinin and is the preferred test for mammals and some avian species. The AGID test is reliable for detection of NP/M antibodies in chickens and turkeys, but not in other avian species. As an alternative, blocking ELISA tests have been developed to detect NP/M antibodies in all avian species.

The HI and NI tests can be used to subtype AI viruses into 15 haemagglutinin and 9 neuraminidase subtypes. Such information is helpful for epidemiological investigations and in categorization of AI viruses.

Poultry can be vaccinated with a variety of AI vaccines including inactivated whole AI virus vaccines, and haemagglutinin expression-based vaccines. Antibodies to the haemagglutinin confer subtype specific protection. Various strategies can be used to differentiate vaccinated from infected birds including serosurveillance in unvaccinated sentinel birds or specific serological tests in the vaccinated birds.

AI virus infection of unvaccinated birds including sentinels is detected by antibodies to the NP/M, subtype specific HA or NA proteins, or NSP. In poultry vaccinated with haemagglutinin expression-based vaccines, antibodies are detected to the specific HA, but not any of the other AI viral proteins. Infection is evident by antibodies to the NP/M or NSP, or the specific NA protein of the field virus. Poultry vaccinated with inactivated whole AI vaccines may develop low titres of antibodies to NSP, but the titre in infected birds will be markedly higher. Alternatively, usage of a vaccine strain with a different NA subtype than the field virus can allow differentiation of vaccinated from infected birds (DIVA) by detection of subtype specific NA antibodies of the field virus. Vaccines used should comply with the standards of the *Terrestrial Manual*.

All flocks with seropositive results should be investigated. Epidemiological and supplementary laboratory investigation results should document the status of NAI infection/circulation for each positive flock.

A confirmatory test should have a higher specificity than the screening test and sensitivity at least equivalent than that of the screening test.

Information should be provided on the performance characteristics and validation of tests used.

1) The follow up procedure in case of positive test results if vaccination is used

In case of vaccinated populations, one has to exclude the likelihood that positive test results are indicative of virus circulation. To this end the following procedure should be followed in the investigation of positive serological test results derived from surveillance conducted on NAI-vaccinated poultry. The investigation should examine all evidence that might confirm or refute the hypothesis that the positive results to the serological tests employed in the initial survey were not due to virus circulation. All the epidemiological information should be substantiated and the results should be collated in the final report.

Knowledge of the type of vaccine used is crucial in developing a serological based strategy to differentiate infected from vaccinated animals.

- a) Inactivated whole AI virus vaccines can use either homologous or heterologous neuraminidase subtypes between the vaccine and field strains. If poultry in the population have antibodies to NP/M and were vaccinated with inactivated whole AI virus vaccine, the following strategies should be applied:
 - i) sentinel birds should remain NP/M antibody negative. If positive for NP/M antibodies, indicating AI virus infection, specific HI tests should be performed to identify H5 or H7 AI virus infection;
 - ii) if vaccinated with inactivated whole AI virus vaccine containing homologous NA to field virus, the presence of antibodies to NSP could be indicative of infection. Sampling should be initiated to exclude the presence of NAIIV by either virus isolation or detection of virus specific genomic material or proteins;
 - iii) if vaccinated with inactivated whole AI virus vaccine containing heterologous NA to field virus, presence of antibodies to the field virus NA or NSP would be indicative of infection. Sampling should be initiated to exclude the presence of NAIIV by either virus isolation or detection of virus specific genomic material or proteins.
- b) Hemagglutinin expression-based vaccines contain the HA protein or gene homologous to the HA of the field virus. Sentinel birds as described above can be used to detect AI infection. In vaccinated or sentinel birds, the presence of antibodies against NP/M, NSP or field virus NA is indicative of infection. Sampling should be initiated to exclude the presence of NAIIV by either virus isolation or detection of virus specific genomic material or proteins.

2) The follow up procedure in case of positive test results indicative of infection for determination of infection due to HPNAI or LPNAI virus

The detection of antibodies indicative of a NAI virus infection as indicated in point a)i) above will result in the initiation of epidemiological and virological investigations to determine if the infections are due to HPNAI or LPNAI viruses.

Virological testing should be initiated in all antibody-positive and at risk populations. The samples should be evaluated for the presence of AI virus, by virus isolation and identification, and/or detection of influenza A specific proteins or nucleic acids (Figure 2). Virus isolation is the gold standard for detecting infection by AI virus and the method is described in the *Terrestrial Manual*. All AI virus isolates should be tested to determine HA and NA subtypes, and *in vivo* tested in chickens and/or sequencing of HA proteolytic cleavage site of H5 and H7 subtypes for determination of classification as HPNAI, LPNAI or LPAI (not notifiable) viruses. As an alternative, nucleic acid detection tests have been developed and validated; these tests have the sensitivity of virus isolation, but with the advantage of providing results within a few hours. Samples with detection of H5 and H7 HA subtypes by nucleic acid detection methods should either be submitted for virus isolation, identification, and *in vivo* testing in chickens, or sequencing of nucleic acids for determination of proteolytic cleavage site as HPNAI or LPNAI viruses. The antigen detection systems, because of low sensitivity, are best suited for screening clinical field cases for infection by Type A influenza virus looking for NP/M proteins. NP/M positive samples should be submitted for virus isolation, identification and pathogenicity determination.

Laboratory results should be examined in the context of the epidemiological situation. Corollary information needed to complement the serological survey and assess the possibility of viral circulation includes but is not limited to:

- a) characterization of the existing production systems;
- b) results of clinical surveillance of the suspects and their cohorts;
- c) quantification of vaccinations performed on the affected sites;
- d) sanitary protocol and history of the affected establishments;
- e) control of animal identification and movements;
- f) other parameters of regional significance in historic NAIV transmission.

The entire investigative process should be documented as standard operating procedure within the epidemiological surveillance programme.

Figure 1 - Schematic representation of laboratory tests for determining evidence of NAI infection through or following serological surveys

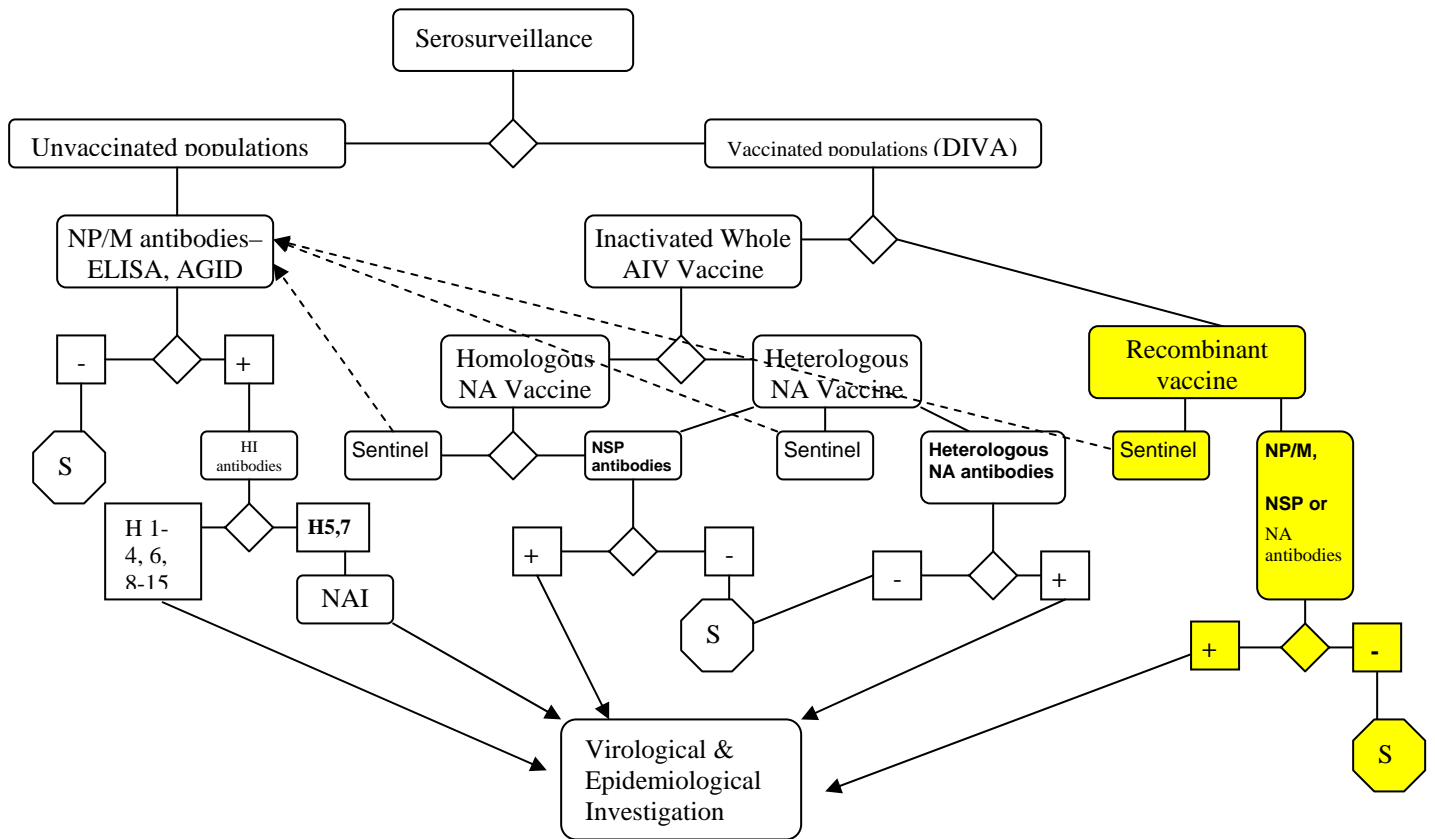
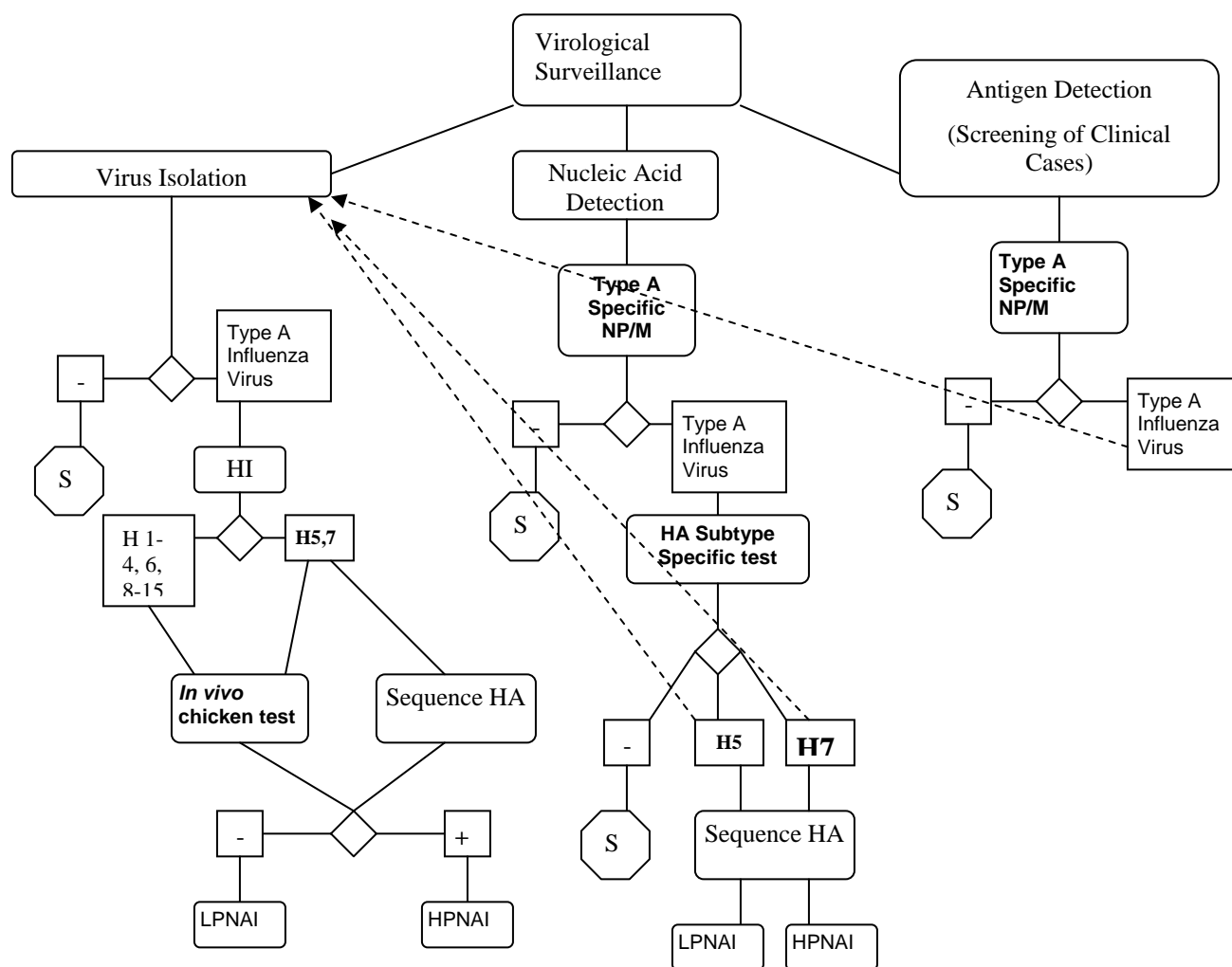


Figure 2. - Schematic representation of laboratory tests for determining evidence of NAI infection using virological methods



The above diagram indicates the tests which are recommended for use in the investigation of poultry flocks.

Key:

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| AGID | Agar gel immunodiffusion |
| DIVA | Differentiating infected from vaccinated animals |
| ELISA | Enzyme-linked immunosorbant assay |
| HA | Haemagglutinin |
| HI | Haemagglutination inhibition |
| NA | Neuraminidase |
| NI | Neuraminidase inhibition |
| NP/M | Nucleoprotein and matrix protein |
| NSP | Nonstructural protein |
| SN | Serum neutralization |

S No evidence of NAIV
